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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07K 14/00, 14/435, 14/705, C12N 5/10, 15/11, 15/63, G01N 33/53, 33/566	A1	(11) International Publication Number: WO 98/29439 (43) International Publication Date: 9 July 1998 (09.07.98)
(21) International Application Number: PCT/US97/23890 (22) International Filing Date: 18 December 1997 (18.12.97) (30) Priority Data: 60/033,851 27 December 1996 (27.12.96) US (71) Applicant (for all designated States except US): MERCK & CO., INC. [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): TAN, Carina [MY/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). SUL-LIVAN, Kathleen [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065 (US). (74) Common Representative: MERCK & CO., INC.; 126 East Lincoln Avenue, Rahway, NJ 07065 (US).		(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: GALANIN RECEPTOR GALR2 AND NUCLEOTIDES ENCODING SAME (57) Abstract <p>A new galanin receptor, GALR2, is described. Also provided are nucleic acids encoding same and various assays to identify ligands particular to said receptor. Ligands so identified are useful for the treatment of obesity, treatment of pain, and treatment of cognitive disorders.</p>		

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TITLE OF THE INVENTION

GALANIN RECEPTOR GALR2 AND NUCLEOTIDES ENCODING
SAME

5 CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable

STATEMENT REGARDING FEDERALLY-SPONSORED R&D

Not applicable

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REFERENCE TO MICROFICHE APPENDIX

Not applicable

FIELD OF THE INVENTION

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This invention relates to a novel galanin receptor,
designated GALR2, to nucleotides encoding it, and to assays which use
it.

BACKGROUND OF THE INVENTION

20

Although first isolated from porcine intestine, galanin is
widely distributed in the central and peripheral nervous system.
Galanin in most species is a 29 amino acid peptide with an amidated
carboxyl terminus. Human galanin is unique in that it is longer, 30
amino acids, and is not amidated. There is strong conservation of the
galanin sequence with the amino terminal fifteen residues being
absolutely conserved in all species. Galanin immunoreactivity and
binding is abundant in the hypothalamus, the locus coeruleus, the
hippocampus and the anterior pituitary, as well as regions of the spinal
cord, the pancreas and the gastrointestinal tract.

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Like neuropeptide Y (NPY), injection of galanin into the
paraventricular nucleus (PVN) of the hypothalamus produces a dose-
dependent increase in feeding in satiated rats. While galanin, like
norepinephrine, enhances carbohydrate ingestion, some studies have
shown that it profoundly increases fat intake. It has been suggested that

galanin shifts macronutrient preference from carbohydrate to fat. The same injections that increase feeding reduce energy expenditure and inhibit insulin secretion. There is enhanced galanin expression in the hypothalamus of genetically obese rats compared with their lean littermate controls. Injection of peptide receptor antagonists into the PVN blocks the galanin-specific induction of increased fat intake. Specific galanin antisense oligonucleotides when injected into the PVN produce a specific decrease in galanin expression associated with a decrease in fat ingestion and total caloric intake while hardly affecting either protein or carbohydrate intake. Thus galanin appears to be one potential neurochemical marker related to the behavior of fat ingestion.

Galanin inhibits cholinergic function and impairs working memory in rats. Lesions that destroy cholinergic neurons result in deficits in spatial learning tasks. While locally administered acetylcholine (ACh) reverses some of this deficit, galanin blocks this ACh-mediated improvement. Evidence from autopsy samples from Alzheimer's disease-afflicted brains suggests an increased galinergic innervation of the nucleus basalis. Thus, if galinergic overactivity contributes to the decline in cognitive performance in Alzheimer's disease, galanin antagonists may be therapeutically useful in alleviating cognitive impairment.

In the rat, administration of galanin intracerebroventricularly, subcutaneously or intravenously increases plasma growth hormone. Infusion of human galanin into healthy subjects also increases plasma growth hormone and potently enhances the growth hormone response to GHRH.

Galanin levels are particularly high in dorsal root ganglia. Sciatic nerve resection dramatically up-regulates galanin peptide and mRNA levels. Chronic administration of galanin receptor antagonists (M35, M15) after axotomy results in a marked increase in self mutilation behavior in rats, generally considered to be a response to pain. Application of antisense oligonucleotides specific for galanin to the proximal end of a transected sciatic nerve suppressed the increase in galanin peptide levels with a parallel increase in autotomy. Galanin injected intrathecally acts synergistically with morphine to produce analgesia, this antinociceptive effect of morphine is blocked by galanin

receptor antagonists. Thus, galanin agonists may have some utility in relieving neural pain.

The actions of galanin are mediated by high affinity galanin receptors that are coupled by pertussis toxin sensitive G_i/G_o proteins to inhibition of adenylate cyclase activity, closure of L-type Ca^{++} channels and opening of ATP-sensitive K^+ channels. Specific binding of ^{125}I -galanin (K_d approximately 1 nM) has been demonstrated in areas paralleling localization of galanin immunoreactivity: hypothalamus, ventral hippocampus, basal forebrain, spinal cord, pancreas and pituitary. In most tissues the amino terminus (GAL 1-15) is sufficient for high affinity binding and agonist activity.

Recently, a galanin receptor cDNA was isolated by expression cloning from a human Bowes melanoma cell line. (Habert-Ortoli, et al. 1994. *Proc. Nat. Acad. Sci., USA* 91: 9780-9783). This receptor, GALR1, is expressed in human fetal brain and small intestine, but little else is known of its distribution. Gal(1-16) is at least 1000 times more active than pGAL(3-29) as an inhibitor of ^{125}I -porcine galanin binding to this receptor transiently expressed in COS cells. It remains to be determined whether this receptor subtype represents the hypothalamic receptor that mediates the galanin specific feeding behavior.

It would be desirable to identify further galanin receptors so that they can be used to further characterize this biological system and to identify galanin receptor subtype selective agonists and antagonists.

SUMMARY OF THE INVENTION

This invention relates to a novel galanin receptor, designated GALR2, substantially free from associated proteins, and to GALR2-like receptors which are at least about 40% homologous and which have substantially the same biological activity. In preferred embodiments of this invention, the GALR2-like receptors are at least about 60%, and more preferably at least about 75%, and even more preferably at least about 85% homologous to a GALR2 receptor. This invention also relates specifically to rat, human and mouse GALR2, substantially free from associated proteins, and to receptors which are at

least about 50% homologous and which have substantially the same biological activity.

Another aspect of this invention are primate and non-primate GALR2 proteins which are encoded by substantially the same nucleic acid sequences, but which have undergone changes in splicing or other RNA processing-derived modifications or mutagenesis-induced changes, so that the expressed protein has a homologous, but different amino acid sequence from the native forms. These variant forms may have different and/or additional functions in human and animal physiology or *in vitro* in cell based assays.

A further aspect of this invention are nucleic acids which encode a galanin receptor or a functional equivalent from rat, human, mouse, swine, or other species. These nucleic acids may be free from associated nucleic acids, or they may be isolated or purified. The nucleic acids which encode a receptor of this invention may be any type of nucleic acid. Preferred forms are DNAs, including genomic and cDNA, although this invention specifically includes RNAs as well. Nucleic acid constructs may also contain regions which control transcription and translation such as one or more promoter regions, termination regions, and if desired enhancer regions. The nucleic acids may be inserted into any known vector including plasmids, and used to transfect suitable host cells using techniques generally available to one of ordinary skill in the art.

Another aspect of this invention are vectors comprising nucleic acids which encode GALR2, and host cells which contain these vectors. Still another aspect of this invention is a method of making GALR2 comprising introducing a vector comprising nucleic acids encoding GALR2 into a host cell under culturing conditions.

Yet another aspect of this invention are assays for GALR2 ligands which utilize the receptors and/or nucleic acids of this invention. Preferred assays of this embodiment compare the binding of the putative GALR2 ligand to the binding of galanin to GALR2.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. is the nucleic acid sequence of rat GALR2 (clone 27A) containing 5' and 3' untranslated regions (SEQ ID NO:1).

FIGURE 2 is the nucleic acid sequence of GALR2 (clone 27A) from initiator Met to termination codon (SEQ ID NO: 2).

FIGURE 3 is a schematic representation of GALR2 (clone 27A) and the nucleic acid and deduced amino acid sequence of GALR2 (clone 27A) (SEQ ID NOS: 3 and 4).

FIGURE 4 is the deduced amino acid sequence of GALR2 (clone 27A) (SEQ ID NO: 5).

FIGURE 5 is a comparison (PileUp alignment) of amino acid sequences for rat GALR1 (SEQ ID NO: 6) and rat GALR2 (SEQ ID NO:7).

FIGURE 6 is the nucleic acid sequence of the cDNA probe used to isolate GALR2 (SEQ ID NO:8).

FIGURE 7 is the DNA sequence of human GALR2 gene (SEQ ID NO:9).

FIGURE 8 is the DNA sequence (open reading frame only) of human GALR2 gene (SEQ ID NO:10).

FIGURE 9 is the deduced amino acid sequence of human GALR2 (SEQ ID NO:11).

FIGURE 10 demonstrates the pharmacology of human and rat GALR2.

FIGURE 11 illustrates G_q or G_s coupled response (pigment dispersion) as well as G_i -coupled response (pigment aggregation).

FIGURE 12 is the DNA sequence of mouse GALR2 gene (SEQ ID NO:12).

FIGURE 13 is the amino acid sequence for mouse GALR2 gene (SEQ ID NO:13).

FIGURE 14 is a comparison of human, rat and mouse GALR1 and GALR2 protein sequences showing strong sequence conservation among members of the GALR gene family.

FIGURE 15 is the RNA expression profile of human GALR2.

FIGURE 16 illustrates the expression of rat GALR2 in the brain.

DETAILED DESCRIPTION OF THE INVENTION

As used throughout the specification and claims, the following definitions apply:

"Substantially free from associated proteins" means that
5 the receptor is at least about 90%, and preferably at least about 95% free from other cell membrane proteins which are normally found in a living mammalian cell which expresses a galanin receptor.

"Substantially free from associated nucleic acids" means
10 that the nucleic acid is at least about 90%, and preferably at least about 95%, free from other nucleic acids which are normally found in a living mammalian cell which naturally expresses a galanin receptor gene.

"Substantially the same biological activity" means that the
15 receptor-galanin binding constant is within 5-fold of the binding constant of GALR2 and galanin, and preferably within 2-fold of the binding constant of GALR2 and galanin.

"Stringent post-hybridizational washing conditions" means
0.1 X standard saline citrate (SSC) at 65°C.

"Standard post-hybridizational washing conditions" means
6 x SSC at 55°C.

20 "Relaxed post-hybridizational washing conditions" means 6 x SSC at 30°C, or 1 to 2 X SSC at 55°C.

"Functional equivalent" means that a receptor which does
not have the exact same amino acid sequence of a naturally occurring
GALR2 protein due to alternative splicing, deletions, mutations, or
25 additions, but retains at least 1%, preferably 10%, and more preferably 25% of the biological activity of the naturally occurring receptor. Such derivatives will have a significant homology with a natural GALR2 and can be detected by reduced stringency hybridization with a DNA
sequence obtained from a GALR2. The nucleic acid encoding a
30 functional equivalent has at least about 60% homology at the nucleotide level to a naturally occurring receptor nucleic acid.

It has been found, in accordance with this invention, that
there is a second galanin receptor, which is designated GALR2. The
35 rat, human and mouse GALR2 sequences are given in FIGURES 4, 9 and 13, respectively, and are referenced in the Examples; however it is to

be understood that this invention specifically includes GALR2 without regard to the species and, in particular, specifically includes rodent (including rat and mouse), rhesus, swine, chicken, cow and human. The galanin 2 receptors are highly conserved throughout species, and one of ordinary skill in the art, given the rat, human and/or mouse sequences presented herein, can easily design probes to obtain the GALR2 from other species.

GALR2 proteins contain various functional domains, including one or more domains which anchor the receptor in the cell membrane, and at least one ligand binding domain. As with many receptor proteins, it is possible to modify many of the amino acids, particularly those which are not found in the ligand binding domain, and still retain at least a percentage of the biological activity of the original receptor. Thus this invention specifically includes modified functionally equivalent GALR2s which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GALR2s which contain modifications and/or deletions in other domains, which are not accompanied by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

The proteins of this invention were found to have structural features which are typical of the 7-transmembrane domain (TM) containing G-protein linked receptor superfamily (GPC-R's or 7-TM receptors). Thus GALR2 proteins make up new members of the GPC-R family of receptors. The intact GALR2 of this invention was found to have the general features of GPC-R's, including seven transmembrane regions, three intra- and extracellular loops, and the GPC-R protein signature sequence. The TM domains and GPC-R protein signature sequence are noted in the protein sequences of the GALR2. Not all regions are required for functioning, and therefore this invention also comprises functional receptors which lack one or more non-essential domains.

Determination of the nucleotide sequence indicated that the GALR2 belongs to the intron-containing class of GPC-R's. Clone 27A, a precursor mRNA terminating in a poly (A) tract, encodes a 1119 bp open reading frame divided into two exons by a single intron of approximately 500 bp (FIGURE 4). Exon 1 encodes the N-terminal extracellular domain through predicted TM-3, while exon 2 encodes the second predicted extracellular loop through the C-terminal intracellular domain. A perfectly conserved splice donor site (G/gt) is found at nucleotide 368 which coincides with the second residue of the G protein-coupled receptor signature aromatic triplet, (D,E) RY.

Removal of the intron indicates that clone 27A encodes a full-length rat galanin receptor polypeptide of 372-amino acids with 7 predicted TM domains, as underlined in FIGURE 4. Searches of nucleic acid and protein sequence databases revealed that the open reading frame sequence is unique and most closely related to rat galanin 1 receptor (GALR1) with 55% nucleic acid and 38% protein sequence identity. An alignment of the protein sequences for rat GALR1 and GALR2 is given in FIGURE 5. Several conserved features ascribed to GPC-R's were also identified in the rat GALR2: the signature aromatic triplet sequence (Glu-Arg-Tyr) adjacent to TM-3, Cys-98 and Cys-153 in the first two extracellular loops capable of disulfide bonding, putative amino-terminal N-glycosylation sites (Asn-Xaa-Ser/Thr), phosphorylation sites in the carboxyl-terminus and the third cytoplasmic loop, and conserved proline residues in TM-4, 5, 6 and 7.

A second cDNA clone was isolated, termed clone 16.6, which does not contain an intron and is therefore a contiguous cDNA containing the complete open reading frame of GALR2. Like clone 27A, Clone 16.6 contains a 5' untranslated region of approximately 500 bp, a contiguous GALR2 open reading frame encoding 7-TM domains (1119 bp), a 3' untranslated region of about 320 bp, and a poly (A) tract. The open reading frame sequence is identical for clones 27A and 16.6 except for nucleotide 109 of the open reading frame (located in predicted TM-1). Clone 27A contains a T while Clone 16.6 contains a C in position 109. Thus, amino acid 37 of the GALR2 protein is phenylalanine in Clone 16.6 and isoleucine in Clone 27A. Both the DNAs of clones 27A and Clone 16.6 form aspects of this invention, as do their respective proteins.

The human GALR2 protein bears strong sequence identity and similarity to the rat GALR2 ortholog. One notable difference between the human and rat forms is the presence of an additional 15 amino acids in the C-terminal intracellular domain of human GALR2.

5 The mouse protein sequence, as well, bears very strong identity and similarity with the GALR gene family.

This invention also relates to truncated forms of GALR2, particularly those which encompass the extracellular portion of the receptor, but lack the intracellular signaling portion of the receptor, and
10 to nucleic acids encoding these truncated forms. Such truncated receptors are useful in various binding assays. Thus this invention specifically includes modified functionally equivalent GALR2s which have deleted, truncated, or mutated N-terminal portions. This invention also specifically includes modified functionally equivalent GALR2s
15 including receptor chimeras which contain modifications and/or deletions in other domains, which are not accompanied by a loss of functional activity.

Additionally, it is possible to modify other functional domains such as those that interact with second messenger effector
20 systems, by altering binding specificity and/or selectivity. Such functionally equivalent mutant receptors are also within the scope of this invention.

Assays which make up further aspects of this invention include binding assays (competition for ¹²⁵I-galanin binding), coupling
25 assays (including galanin-mediated inhibition of forskolin-stimulated adenylate cyclase in cells expressing galanin receptors), measurement of galanin-stimulated calcium release in cells expressing galanin receptors (such as aequorin assays), stimulation of inward rectifying potassium channels (GIRK channels, measured by voltage changes) in
30 cells expressing galanin receptors, and measurement of pH changes upon galanin stimulation of cells expressing galanin receptors as measured with a microphysiometer.

Host cells may be cultured under suitable conditions to produce GALR2. An expression vector containing DNA encoding the
35 receptor may be used for expression of receptor in a recombinant host cell. Recombinant host cells may be prokaryotic or eukaryotic, including but not limited to bacteria such as *E. coli*, fungal cells such as yeast,

mammalian cells including but not limited to cell lines of human, bovine, porcine, monkey and rodent origin, and insect cells including but not limited to *Drosophila*, *Spodoptera*, and silkworm derived cell lines. Cell lines derived from mammalian species which are suitable and which are commercially available include, but are not limited to, L cells L-M(TK⁻) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651), CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C1271 (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

The specificity of binding of compounds showing affinity for the receptor is shown by measuring the affinity of the compounds for cells transfected with the cloned receptor or for membranes from these cells. Expression of the cloned receptor and screening for compounds that inhibit the binding of radiolabeled ligand to these cells provides a rational way for rapid selection of compounds with high affinity for the receptor. These compounds identified by the above assays may be agonists or antagonists of the receptor and may be peptides, proteins, or non-proteinaceous organic molecules. Alternatively, functional assays of the receptor may be used to screen for compounds which affect the activity of the receptor. Such functional assays range from *ex vivo* muscle contraction assays to assays which determine second messenger levels in cells expressing the receptor. The second messenger assays include, but are not limited to, assays to measure cyclic AMP or calcium levels or assays to measure adenyl cyclase activity. These compounds identified by the above assays may be agonists, antagonists, suppressors, or inducers of the receptor. The functional activity of these compounds is best assessed by using the receptor either natively expressed in tissues or cloned and exogenously expressed.

Using the assays of this invention, galanin agonists and antagonists may be identified. A galanin agonist is a compound which binds to the GALR2, such as a galanin mimetic, and produces a cellular response which is at least about equivalent to that of galanin, and which may be greater than that of galanin. Such compounds would be useful in situations where galanin insufficiency causes anorexia, or for treatment of pain.

Also using this embodiment of the assay, galanin antagonists may be identified. A galanin antagonist is a compound which can bind to the GALR2, but produces a lesser response than that of native galanin. Such compounds would be useful in the treatment of obesity.

One assay of this invention is a method of identifying a compound which modulates GALR2 receptor comprising: a) culturing cells expressing the GALR2 receptor in the presence of the compound and b) measuring GALR2 receptor activity or second messenger activity. If desired, the determined activity can be compared to a standard, such as that measured using galanin as the compound. In preferred embodiments, the cells are transformed and express the GALR2 receptor.

The consultant cDNA clone (or shorter portions of, for instance, only 15 nucleotides long) may be used to probe libraries under hybridization conditions to find other receptors which are similar enough so that the nucleic acids can hybridize, and is particularly useful for screening libraries from other species. In this step, one of ordinary skill in the art will appreciate that the hybridization conditions can vary from very stringent to relaxed. Proper temperature, salt concentrations, and buffers are well known.

The following non-limiting Examples are presented to better illustrate the invention.

EXAMPLE 1

A cDNA library from rat hypothalamus was constructed in the plasmid-based mammalian vector pcDNA-3 (InVitrogen, San Diego, CA). Total RNA was isolated from freshly-dissected rat hypothalami (flash-frozen in liquid nitrogen) using the RNagents total RNA isolation kit (Promega Biotech, Madison, WI) with a yield of approximately 0.5 mg from 1 g (wet weight) of hypothalamic tissue. Poly (A)⁺ mRNA was selected using the Poly A tract mRNA Isolation System III (Promega Biotech) with a yield of approximately 6 µg from 0.5 µg total RNA. 3 µg of poly (A)⁺ was then utilized as a template for cDNA synthesis using a kit (Choice Superscript, Life Technologies, Gaithersburg, MD) with both

- random hexamer and oligo (dT)-Not I priming. The double-stranded cDNA was adapted for insertion into the BstXI site of pCDNA-3 using EcoRI/BstXI adapters and transformed by electroporation into the *E.coli* strain HB101. The resulting library contained approximately 750,000
- 5 primary transformants with 90% of the clones containing inserts (average size 1-2 kb). The library (approximately 700,000 cfu) was plated onto LB plates containing ampicillin and chloramphenicol and probed with a approximately 280 bp PCR fragment (SEQ ID NO:8).
- Hybridization was conducted at 32°C for 18 hrs. in 5 X SSPE buffer
- 10 containing 50% formamide, 4 X Denhardt's solution, 0.1% SDS, 10% dextran sulfate, 30 µg/ml sheared salmon-sperm DNA with 2×10^6 cpm/ml of ^{32}P -labeled probe. The probe was radiolabeled by random-priming with $[\alpha]^{32}\text{P}$ -dCTP to a specific activity of greater than 10^9 dpm/µg. The filters were then washed in 1 x SSC, 0.1% SDS at 55°C and
- 15 exposed to film (Kodak X-omat) for 48 hrs. Two independent positive clones were identified (clones 27A and 16.6) and subjected to further analysis.

EXAMPLE 2

20 Sequence Analysis of GALR2

- DNA was prepared from overnight cultures using the Wizard DNA Purification System (Promega Corp., Madison, WI) and subjected to automated sequence analysis using the PRISM Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, CA) on
- 25 an ABI 377 instrument. Initial sequencing primers were complementary to the T7 and SP6 promoter sites in pcDNA-3, additional primers were made complementary to the insert DNA. Database searches (Genbank, EMBL, Swiss-Prot, PIR, dEST, Prosite, dbGPCR), sequence alignments, and analysis of the galanin receptor nucleotide
- 30 and protein sequences were carried out using the GCG Sequence Analysis Software Package (Madison, WI; pileup, peptide structure and motif programs), FASTA and BLAST search programs, and the PC/Gene software suite from Intelligenetics (San Francisco, CA; protein analysis programs).

EXAMPLE 3

Construction of a Vector for Expression of GALR2

Five μ g of the mammalian expression vector pCI.neo
5 (Promega Biotech, Madison WI) was digested with 20 units of EcoRI for 2
hours at 37°C. The digest was then treated with calf intestinal
phosphatase and then electrophoresed on 1% Seaplaque gel in 1X TAE
buffer and the band corresponding to linearized vector was cut out. DNA
was recovered from the slice after melting at 65°C using the Promega
10 Wizard PCR system (Promega Biotech). DNA was quantitated by
electrophoresis with standards on a 1% TBE gel. 100 ng of the 2200 bp
EcoRI insert (including the intron) from pCDNA-3/27A was ligated to 50
ng of the vector pCI.neo in a 10 ml reaction at room temperature for 1
hour. 1 μ l of this ligation mixture was used to transform 50 μ l competent
15 DH5a cells (Life Technologies). Clones in the correct orientation were
selected following a digest with BamHI. Transfection-quality DNA was
then prepared using the Qiagen Maxi protocol (Qiagen, Chatsworth,
CA). Mammalian COS-7 cells were transfected by electroporation. COS-
7 cells (1×10^7) were suspended in 0.85 ml of Ringers' buffer and 15 mg of
20 the pCI.neo/27A clone was added to a 0.4 mm electroporation cuvette
(Bio-Rad, Hercules, CA). Current was applied (960 μ F, 260 V) using a
Bio-Rad Electroporator device and the cells were transferred to a T-180
flask (Corning). Expression was allowed to proceed for 72 hrs.

25

EXAMPLE 4

Pharmacology of GALR2

Membranes were prepared from transfected cells following
dissociation in enzyme-free dissociation solution (Specialty Media,
Lavallette, NJ) by disruption in a Dounce homogenizer in ice-cold
30 membrane buffer (10 mM Tris, pH 7.4, 10 mM PMSF, 10 μ M
phosphoramidon, and 40 μ g/ml bacitracin). After a low speed (1100 x g
for 10 min. at 4°C) and a high speed centrifugation (38,700 x g for 15 min.

at 4°C), membranes were resuspended in buffer and protein concentration determined (Bio-Rad assay kit). Binding of ^{125}I -human galanin (specific activity of 2200 Ci/mmol, DuPont NEN) was measured in membranes using a buffer of 25 mM Tris pH 7.4, 0.5% BSA, 2 mM MgCl_2 , 40 $\mu\text{g/ml}$ bacitracin, 4 $\mu\text{g/ml}$ phosphoramidon, and 10 μM leupeptin in a total volume of 250 μl . 70 pM ^{125}I -human galanin was used. Reactions were initiated by the addition of membranes and the incubation was allowed to proceed at room temperature for 1 hour. Non-specific binding was defined as the amount of radioactivity remaining bound in the presence of 1 μM cold galanin. In competition studies various concentrations of peptides (hGal, pGal, hGal(1-16), rGAL(2-29), rGAL(3-29), hGal (1-19) or chimeric peptides (C7, M15, M40, M35) were included along with ^{125}I -hGal (70 pmol). Incubations were terminated by rapid filtration through GF/C filters which had been presoaked with 0.1% polyethylamine using a TOMTEC (Orange, CT) cell harvester. The results were analyzed using the Prism software package (GraphPad, San Diego, CA). Shown in the table below is the ligand binding profiles of both rat GALR1 and rat GALR2 proteins (clone 27A shown; clone 16.6 gave similar results). The K_D for binding of ^{125}I -labeled human galanin against rat GALR2 was 0.2 nM.

		IC50 (nM)	
		rat GALR1	rat GALR2 (clone 27A)
25	pig Galanin	0.06	0.46
	human Galanin	0.07 \pm 0.01	1.3 \pm 0.5
	rat Gal (2-29)	7.2	2.9 \pm 1.3
	rat Gal (3-29)	>1000	>1000
	human Gal (1-19)	0.86	
30	pig Gal (1-16)	0.27 \pm 0.18	3.0
	galantide(M15)	1.0 \pm 1.1	28 \pm 3.5
	C7	4.9 \pm 3	23 \pm 13
	M40	0.01	1.9 \pm 0.14
	M35	0.9 \pm 0.6	0.43 \pm 0.18

EXAMPLE 5

Expression of rat GALR2

In situ hybridization was conducted to map the distribution of GALR2 mRNA in rat brain using a ³²P-labeled GALR2 ORF fragment as a hybridization probe; see O'Dowd, B. F. et al. 1995 Genomics 28:84-91. Specific hybridization was detected in a number of brain nuclei and regions, most notably supra-, pre-(PMD/ PMV), med- and lateral mammillary nuclei, the dentate gyrus (DG), cingulate gyrus (CG), posterior hypothalamic (PH), supraoptic and arcuate nuclei (Arc) as shown in Figure 16. Both frontal and parietal cortical regions were also labeled.

Clone Isolation of Human GALR2: Cloning of Partial GalR2 gene by degenerate PCR.

Human genomic DNA was amplified by PCR using degenerate oligonucleotides designed based on the sequences encoding transmembranes (TM) regions TM3 (P1: 5' CTG ACC GYC ATG RSC ATT GAC SGC TAC, SEQ ID NO:14, wherein Y = C or T, R=A or G, S = C or G) and TM7 (P2: 5'-GGG GTT GRS GCA GCT GTT GGC RTA, SEQ ID NO:15) of somatostatin receptors and the receptor encoded by the somatostatin-related gene, SLC-1. The PCR conditions were as follows: denaturation at 95°C for 1 min, annealing at either 55°C, 45°C, or 38 °C for 1 min and extension at 72°C for 2.5 min for 30 cycles, followed by a 7 min extension at 72°C. The resultant PCR products were phenol/chloroform extracted, precipitated with ethanol, phosphorylated with T4 polynucleotide kinase, and blunt-ended with Klenow enzyme. Subsequently, they were electrophoresed on a 0.5% low-melting point agarose and a fragment of the expected size was subcloned into the EcoRV site of pBluescript SK(-) (Stratagene, La Jolla, CA). Colonies were selected, plasmid DNA was purified, and the inserts sequenced.

EXAMPLE 6

Gene Sequence and Structure: Cloning and sequencing of Human GalR2 Genomic DNA.

DNA fragments radiolabelled with [32P]dCTP by nick translation (Amersham) were used as a probe to screen a EMBL3 SP6/T7 human genomic library (Clontech, Palo Alto, CA). Positive phage clones were plaque purified, DNA was prepared, restriction enzyme digested, electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library, as described by Marchese et al, 1994 [Genomics 23, 609-618]. Positive phage were subcloned by digesting phage DNA, and subcloning the resultant fragment into the pBluescript vector. The DNA sequence of the clone was determined using standard methods on an ABI 372 automated sequencer (Perkin-Elmer-Applied Biosystems, Foster City, CA). As shown in FIGURE 7, the sequence determined shows a gene with a total of two exons interrupted by an 1800 bp intron. The deduced amino acid sequence (FIGURE 9) of the complete open reading frame (FIGURE 8) gives a protein of 387 amino acids with features typical of G protein-coupled receptors including 7 transmembrane alpha helical domains. Figure 14 shows an alignment of GALR1 and GALR2 protein sequences with the seven transmembrane domains underlined. The human GALR2 protein bears strong sequence identity and similarity to the rat GALR2 ortholog. One notable difference between the human and rat forms is the presence of an additional 15 amino acids in the C-terminal intracellular domain of human GALR2.

EXAMPLE 7

Receptor Expression: Human and Rat GALR2: Construction of Human GalR2 Expression Plasmid

The human GalR2 expression construct was assembled from the human genomic clone by PCR. Each exon was PCR amplified using standard conditions. The primers for exon I were: Forward, Exon

I (5' - CCG GAA TTC GGT ACC ATG AAC GTC TCG GGC TGC CC - 3'; SEQ ID NO:16) and Reverse, Exon I (5' - GGT AGC GGA TGG CCA GAT ACC TGT CTA GAG AGA CGG CGG CC - 3'; SEQ ID NO:17). The primers for exon II were: Forward, Exon II (5' - GGC CGC CGT CTC TCT AGA CAG GTA TCT GGC CAT CCG CTA CC - 3'; SEQ ID NO:18) and Reverse, Exon II (5' - GGC CGC CGT CTC TCT AGA CAG GTA TCT GGC CAT CCG CTA CC - 3'; SEQ ID NO:19). PCR products were subcloned in to pBluescript and sequenced. Exon I product was subcloned into the EcoRI and XbaI sites of plasmid pCINeo (Promega, Madison, WI). Exon II was then cloned into the XbaI site and the orientation determined by appropriate restriction digests and DNA sequencing.

EXAMPLE 8

15 Radioligand binding assay

Plasmid DNA was prepared using the Qiagen Maxi protocol (Qiagen, Chatsworth, CA) and transfected into COS-7 cells by electroporation. Briefly, 0.85 μ l COS-7 cells in Ringers' buffer (1.2×10^7 /ml) and 20 μ g of DNA were mixed in a 0.4 mm electroporation cuvette (Bio-Rad, Hercules, Ca) and current (960 μ F, 260 V) was applied using a Bio-Rad Electroporator device. Cells were transferred to a T-180 flask (Corning) with fresh media and expression was allowed to proceed for 72 hrs. Membranes were prepared from transfected cells following disruption in enzyme-free dissociation solution (Specialty Media, Lavallette, NJ) in a Dounce homogenizer in ice-cold membrane buffer (10 mM Tris, pH 7.4, 10 mM PMSF, 10 μ M phosphoramidon, and 40 μ g/ml bacitracin). After a low speed (1100 x g, 10 min. at 4°C) and a high speed centrifugation (38,700 x g for 15 min. at 4°C), membranes were suspended in buffer and the protein concentration determined (Bio-Rad assay kit). Binding of 125 I-human galanin (sp. act = 2200 Ci/mmol, DuPont NEN) was measured in membranes using a buffer of 25 mM Tris pH 7.4, 0.5% BSA, 2 mM $MgCl_2$, 40 μ g/ml bacitracin, 4 μ g/ml phosphoramidon, and 10 μ M leupeptin in a total volume of 0.25 ml. 70 pm 125 I-human galanin was used. Reactions were initiated by the

addition of membranes and the incubation was allowed to proceed at room temperature for 1 hour. Non-specific binding was defined as the amount of membrane bound radioactivity remaining in the presence of 1 μ M cold galanin. In competition studies various concentrations of peptides (hGal, pGal, hGal(1-16), rGAL(2-29), rGAL(3-29), hGal (1-19) or chimeric peptides (C7, M15, M40, M35) were included along with 125 I-hGal (70 pmol). Incubations were terminated by rapid filtration through GF/C filters which had been presoaked with 0.1% polyethylamine using a TOMTEC (Orange, CT) cell harvester. The results were analyzed using the Prism software package (GraphPad, San Diego, CA).

Recombinant expression of human GALR2 binding sites in transiently transfected COS-7 permitted the determination of pharmacology of the cloned receptor. 125 I-human galanin bound to the cloned GALR2 receptor with high affinity in a saturable and specific manner with a K_D of 5 nM. As summarized in Figure 10, competition of 125 I-human galanin with a variety of galanin-derived peptides and chimeric peptide antagonist/partial agonists showed that the human GALR2 receptor has a similar pharmacology of binding to that of the rat GALR2.

EXAMPLE 9

Functional Characterization: Post-receptor signalling mechanism Frog melanophore assay

Growth of *Xenopus laevis* melanophores and fibroblasts was performed as described previously (Potenza, M.N. et al, 1992, *Pigment Cell Res.* 3:38-43). Briefly, melanophores were grown in fibroblast-conditioned growth medium. The fibroblast-conditioned growth medium was prepared by growing fibroblasts in 70% L-15 medium (Sigma), pH 7.3, supplemented with 20% heat-inactivated fetal bovine serum (Gibco), 100 μ g/ml streptomycin, 100 units/ml penicillin and 2 mM glutamine at 27.5°C. The medium from growing fibroblasts was collected, passed through a 0.2 μ m filter (fibroblast-conditioned growth medium) and used to culture melanophores at 27.5°C.

Plasmid DNA was transiently transfected into melanophores by electroporation using a BTX ECM600 electroporator (Genetronics, Inc. San Diego, CA). Melanophores were incubated in the presence of fresh fibroblast-conditioned frog medium for 1 hour prior to harvesting of cells. Melanophore monolayers were detached by trypsinization (0.25% trypsin, JHR Biosciences), followed by inactivation of the trypsin with fibroblast-conditioned frog medium. The cells were collected by centrifugation at $200 \times g$ for 5 minutes at 4°C . Cells were washed once in fibroblast conditioned frog medium, centrifuged again and resuspended at 5×10^6 cells per ml in ice cold 70% PBS pH 7.0. 400 μl aliquots of cells in PBS were added to prechilled eppendorf tubes containing 2 μg of pcIneo:human Galanin 2 receptor plasmid DNA mixed with control receptor cDNA and naked vector DNA for a total of 20 μg DNA (2 μg each of pcDNA1amp:cannabinoid 2 and pcDNA3:thromboxane A2 receptor plasmid DNA, and 18 μg of pcDNA3.1 plasmid DNA in 40 μl total volume, or 2 μg each of pcDNA1amp: cannabinoid 2 and pcDNA3:thromboxane A2 receptor plasmid DNA, and 20 μg of pcDNA3.1 plasmid DNA in 40 μl total volume, as a control). Samples were incubated on ice for 20 min, and mixed every 7 minutes. Cell and DNA mixes were transferred to prechilled 2 mM gap electroporation cuvettes (BTX) and electroporated with the following settings: capacitance of 325 microfarad, voltage of 450 volts and resistance of 720 ohms. Immediately following electroporation, cells were mixed with fibroblast-conditioned frog medium (7.85 mls per cuvette) and plated onto flat bottom 96 well microtiter plates (NUNC). Electroporations from multiple cuvettes were pooled together prior to plating to ensure homogenous transfection efficiency. On the day following transfection, medium was removed and fresh fibroblast-conditioned frog medium was added to the melanophore monolayer and cell were incubated at 27°C .

Cells were assayed for receptor expression 2 days following transfection in 96-well plate format. On the day of ligand stimulation, medium was removed by aspiration and cells were washed with 70% L-15 containing 15 mM HEPES pH 7.3 (Sigma). Assays were dividing into two separate parts in order to examine Gs/Gq functional coupling which results in pigment dispersion in melanophores, or Gi functional coupling which results in pigment aggregation. For Gs/Gq functional coupling responses, assays were performed as follows. Cells were

incubated in 100 μ l of 70% L-15 containing 15 mM HEPES for 1 hour in the dark at room temperature, and then incubated in the presence of melatonin (2 nM final concentration) for 1 hour in the dark at room temperature to induce pigment aggregation. Initial absorbance at 600 nM was measured using a Bio-Tek Elx800 Microplate reader (ESBE Scientific) prior to addition of ligand. Human galanin (Peninsula) was added in duplicate wells, samples were mixed and incubated in the dark at room temperature for 1 hour, after which the final absorbance at 600 nm was determined. For Gi coupled responses, cell monolayers were incubated in the presence of 100 μ l of 70% L-15 containing 2% fibroblast-conditioned growth medium, 2 mM glutamine, 100 ug/ml streptomycin, 100 units/ml penicillin and 15 mM HEPES for 15 minutes in the dark at room temperature to preset the cells to dispersion. After initial absorbance at 600 nM was determined, human galanin was added to cell monolayers, samples were mixed, incubated in the dark for 1.5 hour at room temperature and then final absorbances were determined. Absorbance readings were converted to transmission values in order to quantitate pigment dispersion using the following formula: $1 - T_f/T_i$, where T_i = the initial transmission at 600 nm and T_f = the final transmission at 600 nm. Pigment aggregation was quantitated using the following formula: $A_f/A_i - 1$, where A_f = final absorbance at 600 nm and A_i is initial absorbance at 600 nm.

To determine whether the human GALR2 could be functionally expressed in melanophores, the expression plasmid pcIneo:hGALR2 was transiently transfected by electroporation into melanophores followed by stimulation of the transfected cells with human galanin. Increasing doses of galanin resulted in a dose-dependent dispersion of pigment in human GALR2-transfected melanophores, in contrast to control vector-transfected cells (FIGURE 11). The apparent EC₅₀ for human galanin in pcIneo:hGALR2-transfected melanophores was 20 nM, in general agreement with specific ¹²⁵I-human galanin binding in pcIneo:hGALR2-transfected COS-7 cells (IC₅₀ ~ 4 nM). The dispersion of pigment in the melanophore has been previously shown to occur either through G α s coupling and stimulation of adenylyl cyclase or through G α q coupling and mobilization of calcium.

There was no detectable aggregation of the pigment in either the pcIneo:hGALR2- or mock-transfected melanophores following incubation in the presence of 0.001 - 1000 nM human galanin. This result suggests that the hGALR2 does not couple to G α i-mediated signaling pathways.

EXAMPLE 10

Aequorin bioluminescence assay

Measurement of GALR2 expression in the aequorin-expressing stable reporter cell line 293-AEQ17 (Button, D et al, 1993 "Aequorin-expressing mammalian cell lines used to report Ca²⁺ mobilization" *Cell Calcium* 14:663-671) was performed using a Luminoskan RT luminometer (Labsystems Inc., Gaithersburg, MD) controlled by custom software written for a Macintosh PowerPC 6100. 293-AEQ17 cells (8 x 10⁵ cells plated 18 hrs. before transfection in a T75 flask) were transfected with 22 μ g of rat or human GALR2 plasmid DNA: 264 μ g lipofectamine. Following approximately 40 hours of expression the apo-aequorin in the cells was charged for 4 hours with coelenterazine (10 μ M) under reducing conditions (300 μ M reduced glutathione) in ECB buffer (140 mM NaCl, 20 mM KCl, 20 mM HEPES-NaOH [pH=7.4], 5 mM glucose, 1 mM MgCl₂, 1 mM CaCl₂, 0.1 mg/ml bovine serum albumin). The cells were harvested, washed once in ECB medium and resuspended to 500,000 cells/ml. 100 μ l of cell suspension (corresponding to 5x10⁴ cells) was then injected into the test plate, and the integrated light emission was recorded over 30 seconds, in 0.5 second units. 20 mL of lysis buffer (0.1% final Triton X-100 concentration) was then injected and the integrated light emission recorded over 10 seconds, in 0.5 second units. The "fractional response" values for each well were calculated by taking the ratio of the integrated response to the initial challenge to the total integrated luminescence including the Triton-X100 lysis response.

The aequorin bioluminescence assay is a reliable test for identifying G protein-coupled receptors which couple through the G α protein subunit family consisting of G α q and G α 11 which leads to the

activation of phospholipase C, mobilization of intracellular calcium and activation of protein kinase C. Based on the above melanophore data for GALR2, utilization of the aequorin bioluminescence assay permitted the discrimination between the two possibilities for the primary intra-

5 cellular signaling mechanism for GALR2, namely G α s coupling and stimulation of adenylyl cyclase or G α q coupling and mobilization of calcium. Expression of human or rat GALR2 in the aequorin-expressing 293 cell line (293-AEQ17) gave a dose-dependant increase in aequorin bioluminescence in response to challenge by galanin and

10 several related peptides. Transfection of human GALR1, which signals through G α i and the inhibition of adenylyl cyclase, gave no galanin-dependant increase in aequorin bioluminescence. Responses observed for human or rat GALR2 activation were saturable and the rank order of potency was similar to that observed for competition studies for ¹²⁵I-

15 human galanin binding. EC₅₀'s, given in nM for the human GALR2 (results were similar for the rat GALR2 ortholog) were: human galanin, 32; rat galanin, 12; rat galanin (2-29), 31; rat galanin (3-29) >10,000; M35, 44; M40, 8.8. Of interest to note is that the galanin chimeric peptide antagonists (M35 and M40), thought by some to be pure antagonists on

20 the GALR1 receptor, appear to be partial agonists on the GALR2 receptor. These data indicate that the primary signaling mechanism for GALR2 is through the phospholipase C/protein kinase C pathway, in contrast to GALR1, which communicates its intracellular signal by inhibition of adenylyl cyclase through G α i. In addition, while binding

25 and activation of the rat and human GALR2 receptor by galanin is of high affinity and potency, rat or human GALR1 binds and is activated by galanin at a 10-30 fold lower concentration. This observation points to the existence of other undiscovered naturally-occurring ligand systems that may be agonists at the GALR2 receptor.

30

EXAMPLE 11

RNA Expression profile of Human GalR2

Northern blotting analysis was utilized to assess the tissue specificity of human GALR2 mRNA expression. As shown in FIGURE 5 15, modest expression (indicated by one "+") is seen in a variety of brain regions and peripheral tissues, as observed for the rat ortholog of GALR2. The most prevalent transcript size is ~2.2 kb with a band of ~1.5 kb observed in spleen, thymus and prostate. Tissues with significantly higher expression levels (indicated by two or three "+") were placenta, 10 thymus and prostate.

EXAMPLE 12

Chromosome Localization of Human GalR2 Gene

Fluorescence *in situ* hybridization (FISH) of metaphase 15 spread chromosomes derived from human lymphocytes together with DAPI banding patterns was used to map hGalR2 to its chromosome, as described (Heng, H. H. Q. and Tsui, L.-C. *Modes of DAPI banding and simultaneous in situ hybridization*. Chromosoma 102:325-332). FISH 20 data localize the receptor gene to human chromosome 17q25.

EXAMPLE 13

Mouse GALR2: Clone Isolation: Cloning of Mouse GalR2 Genomic Clone

DNA fragments from the Human GalR2 gene were radiolabelled with [32P]dCTP by random octomer labeling (Gibco BRL) 25 and used as a probe to screen a mouse 129sv genomic library (Stratagene). Positive phage clones were plaque purified, DNA was prepared, restriction enzyme digested, electrophoresed on an agarose gel, transferred to nylon membrane, and hybridized with the same probe used to screen the library. A positive NotI fragment was subcloned into 30 pBluescript (Stratagene).

EXAMPLE 14

Gene Sequence and Structure

- DNA sequence encoding the complete ORF for mouse
- 5 GALR2 (SEQ ID NO:12) is shown in Figure 12. A single intron of 1060 bp divides the ORF into two exons. Removal of the intron allows for conceptual translation to give the predicted GALR2 polypeptide of 371 amino acids (SEQ ID NO:13) as shown in Fig. 13. Compared to both the human and rat orthologs, the mouse protein sequence bears strong
- 10 identity (85 % and 96 % respectively).

WHAT IS CLAIMED:

1. Galanin receptor 2 (GALR2), substantially free from associated proteins, or a GALR2-like receptor, wherein the GALR2-like receptor shares at least about 40% homology to GALR2 and has substantially the same biological activity.
2. A GALR2-like receptor according to Claim 1, which shares at least about 50% homology to a GALR2.
3. A GALR2-like receptor according to Claim 1, which shares at least about 75% homology to a GALR2.
4. A GALR2-like receptor according to Claim 1, which shares at least about 85% homology to a GALR2.
5. Rat GALR2 in accordance with Claim 1.
6. GALR2 according to Claim 1 which is SEQ ID NO:5.
7. GALR2 according to Claim 1 which has the sequence of Clone 16.6.
8. A nucleic acid, substantially free from associated nucleic acids, which encodes a GALR2 or a GALR2-like receptor which is at least about 40 % homologous to GALR2 and which has substantially the same biological activity.
9. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 50% homology to a GALR2.
10. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 75% homology to a GALR2.

11. A nucleic acid encoding a GALR2-like receptor according to Claim 8, wherein the GALR2-like receptor shares at least about 85% homology to human GALR2.
- 5 12. A nucleic acid according to Claim 8 which is DNA.
13. A vector comprising the nucleic acid of Claim 8.
14. A host cell comprising the nucleic acid of Claim 8.
- 10 15. A method of determining if a compound is a GALR2 ligand comprising contacting the compound and GALR2 and determining if binding occurs.
- 15 16. A method of identifying a compound that modulates GALR2 receptor activity, comprising:
- (a) culturing cells expressing GALR2 receptor in the presence of the compound; and
- (b) measuring GALR2 receptor activity or second messenger activity.
- 20 17. A method according to Claim 16 wherein the cells are transformed to express a GALR2 receptor.

10	20	1/26	30	40
<hr/>				
CGCTCCCTCC	ACACCTCCAG	GGGCAGTGAG	CCACTCAAGT	40
CTAAAGCAGA	GCGAGTCCCA	GGA CTTGAGC	GCGGGAAGCG	80
AATGGAGTCA	GGGTCAATTCG	ATTGCACCTC	TCTCGGCTGC	120
GGGCCGGAGC	GGGGTACCAT	CCTACACTCT	GGGTGCTCCC	160
TCCTCCTCCC	GTCCCCCGCG	CACCCCTGCC	CTGGCTCCTG	200
210	220	230	240	
<hr/>				
GAGCTCGGCA	GTCTCGCTGG	GGCGCTGCAG	CGAGGGAGCA	240
GCGTGCTCAC	CAAGACCCGG	ACAGCTGCGG	GAGCGGCGTC	280
CACTTTGGTG	ATACCATGAA	TGGCTCCGGC	AGCCAGGGCG	320
CGGAGAACAC	GAGCCAGGAA	GGCGGTAGCG	GCGGCTGGCA	360
GCCTGAGGCG	GTCTTGTAC	CCCTATTTT	CGCGCTCATC	400
410	420	430	440	
<hr/>				
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TGCTGCTGCG	CGGCGGCCAG	GCGGTCAGCA	CCACCAACCT	480
GTTTCATCCTC	AACCTGGGCG	TGGCCGACCT	GTGTTTCATC	520
CTGTGCTGCG	TGCCTTTCCA	GGCCACCATC	TACACCCTGG	560
ACGACTGGGT	GTTCGGCTCG	CTGCTCTGCA	AGGCTGTTCA	600
610	620	630	640	
<hr/>				
TTTCCTCATC	TTTCTCACTA	TGCACGCCAG	CAGCTTCACG	640
CTGGCCGCCG	TCTCCCTGGA	CAGGTAAAGG	ACCCAGAAAG	680
AAACATCCAG	TATGCCCCGA	GGGATCTTGA	CTGGAAAAGA	720
CTGAATCCTG	GTCTGGTGAC	CTTAGTTCCC	TGCCCTTTCA	760
CATCACTTGG	ACATTCCCAC	AGAAGAGCGG	TGAAGAGGCG	800
810	820	830	840	
<hr/>				
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GTGCGTCCTG	AGTACGCTGG	AGGGACTCAC	AAAATTTTCAG	880
CTTTCTTTAG	GAGTTTCCTT	GCTGTAGTTT	GACCCAAGTC	920
TTCTCCAGGT	TTCTGTCAGA	ACCTCAGGCA	TGAGGGGATCT	960
GCCTCCCCTG	GTTGTCACCA	GAGGATAACA	ATCACTGCCC	1000
1010	1020	1030	1040	
<hr/>				
CCAGAAATCC	AGACAGATTC	TACAACTTTT	AGTCTTCGGT	1040
GTTTTGGGGG	TGCCCCCTTCA	CGTGGAGTAG	GTCGGTGGCC	1080
ACATTCCAG	GAGTGACAAT	AGCCTAGCAG	TGAATCCTCT	1120
CGCTTAGCTG	ATGCCCCCCC	ACTGTCCCCA	CAGGTATCTG	1160
GCCATCCGCT	ACCCGCTGCA	CTCCCGAGAG	TTGCGCACAC	1200

FIG. 1A

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1210	1220	1230	1240
CTCGAAACGC	GCTGGCCGCC	ATCGGGCTCA	TCTGGGGGCT 1240
AGCACTGCTC	TTCTCCGGGC	CCTACCTGAG	CTACTACCGT 1280
CAGTCGCAGC	TGGCCAACCT	GACAGTATGC	CACCCAGCAT 1320
GGAGCGCACC	TCGACGTCGA	GCCATGGACC	TCTGCACCTT 1360
CGTCTTTAGC	TACCTGCTGC	CAGTGCTAGT	CCTCAGTCTG 1400

1410	1420	1430	1440
ACCTATGCGC	GTACCCTGCG	CTACCTCTGG	CGCACAGTCG 1440
ACCCGGTGAC	TGCAGGCTCA	GGTTCCCAGC	GCGCCAAACG 1480
CAAGGTGACA	CGGATGATCA	TCATCGTGGC	GGTGCTTTTC 1520
TGCCTCTGTT	GGATGCCCCA	CCACGCGCTT	ATCCTCTGCG 1560
TGTGGTTTGG	TCGCTTCCCG	CTCACGCGTG	CCACTTACGC 1600

1610	1620	1630	1640
GTTGCGCATC	CTTTCACACC	TAGTTTCCTA	TGCCAACTCC 1640
TGTGTCAACC	CCATCGTTTA	CGCTCTGGTC	TCCAAGCATT 1680
TCCGTAAAGG	TTTCCGCAAA	ATCTGCGCGG	GCCTGCTGCG 1720
CCCTGCCCCG	AGGCGAGCTT	CGGGCCGAGT	GAGCATCCTG 1760
GCGCCTGGGA	ACCATAGTGG	CAGCATGCTG	GAACAGGAAT 1800

1810	1820	1830	1840
CCACAGACCT	GACACAGGTG	AGCGAGGCAG	CCGGGCCCCT 1840
TGTCCCACCA	CCCGCACTTC	CCAAGTGCAC	AGCCTCGAGT 1880
AGAACCCTGG	ATCCGGCTTG	TAAAGGACC	AAAGGGCATC 1920
TAACAGCTTC	TAGACAGTGT	GGCCCGAGGA	TCCCTGGGGG 1960
TTATGCTTGA	ACGTTACAGG	GTTGAGGCTA	AAGACTGARG 2000

2010	2020	2030	2040
ATTGATTGTA	GGGAACCTCC	AGTTATTAAA	CGGTGCGGAT 2040
TGCTAGAGGG	TGGCATAGTC	CTTCAATCCT	GGCACCCGAA 2080
AAGCAGATGC	AGGAGCAGGA	GCAGGAGCAA	AGCCAGCCAT 2120
GGAGTTTGAG	GCCTGCTTGA	ACTACCTGAG	ATCCAATAAT 2160
AAAACATTTT	ATATGCTGTG	AAAAAAAAAA	AAAAAAAAAA 2200

FIG. 1B

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10 20 30 40
ATGAATGGCT CCGGCAGCCA GGGCGCGGAG AACACGAGCC 40
AGGAAGGCGG TAGCGGCGGC TGGCAGCCTG AGGCGGTCTT 80
TGTACCCCTA TTTTTCGCGC TCATCTTCCT CGTGGGCACC 120
GTGGGCAACG CGCTGGTGCT GGGGTGTCTG CTGCGCGGCG 160
GCCAGGCGGT CAGCACCACC AACCTGTTCA TCCTCAACCT 200
210 220 230 240
GGGCGTGGCC GACCTGTGTT TCATCCTGTG CTGCGTGCCT 240
TTCCAGGCCA CCATCTACAC CCTGGACGAC TGGGTGTTCG 280
GCTCGCTGCT CTGCAAGGCT GTTCATTTC TCATCTTCTT 320
CACTATCCAC GCCAGCAGCT TCACGCTGGC CGCCGTCTCC 360
CTGGACAGGT AAAGGACCCA GAAAGAAACA TCCAGTATGC 400
410 420 430 440
CCGGAGGGAT CTTGACTGGA AAAGACTGAA TCCTGGTCTG 440
GTGACCTTAG TTCCCTGCCC TTTCACATCA CTGGGACATT 480
CCCACAGAAG AGCGGTGAAG AGGCGGTGGT CCTTATCTCT 520
CTCTGGTTTC CACTGAGTGC AACATGTGCG TCCTGAGTAC 560
GCTGGAGGGA CTCACAAAAT TTCAGCTTTC TTTAGGAGTT 600
610 620 630 640
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TCAGAACCTC AGGCATGAGG GATCTGCCCT CCCTGGTGTG 680
CACCAGAGGA TAACAATCAC TGCCCCCAGA AATCCAGACA 720
GATTCTACAA CTTTGTAGTCT TCGGTGTFTT GGGGGTGCCC 760
CTTCACGTGG AGTAGGTGGG TGGCCACATT CCCAGGAGTG 800
810 820 830 840
ACAATAGCCT AGCAGTGAAT CCTCTCGCTT AGCTGATGCC 840
CCCCCACTGT CCCCACAGGT ATCTGGCCAT CCGCTACCCG 880
CTGCACTCCC GAGAGTTGGC CACACCTCGA AACGGGCTGG 920
CCGCCATCGG GCTCATCTGG GGGCTAGCAC TGCTCTTCTC 960
CGGGCCCTAC CTGAGCTACT ACCGTCAGTC GCAGCTGGCC 1000

FIG. 2A

SUBSTITUTE SHEET (RULE 26)

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1010 1020 1030 1040
AACCTGACAG TATGCCACCC AGCATGGAGC GCACCTCGAC 1040
GTCGAGCCAT GGACCTCTGC ACCCTCGTCT TTAGCTACCT 1080
GCTGCCAGTG CTAGTCTCA GTCTGACCTA TGCGCGTACC 1120
CTGCGCTACC TCTGGCGCAC AGTCGACCCG GTGACTGCAG 1160
GCTCAGGTC CCAGCGCGCC AAACGCAAGG TGACACGGAT 1200

1210 1220 1230 1240
GATCATCATC GTGGCGGTGC TTTTCTGCCT CTGTTGGATG 1240
CCCCACCACG CGCTTATCCT CTGCGGTGGG TTGGTCCCT 1280
TCCCGCTCAC GCGTGCCACT TACGCGTTGC GCATCCTTTC 1320
ACACCTAGTT TCCTATGCCA ACTCCTGTGT CAACCCCATC 1360
GTTTACGCTC TGGTCTCCAA GCATTTCCGT AAAGGTTTCC 1400

1410 1420 1430 1440
GCAAAATCTG CGCGGGCCTG CTGCGCCCTG CCCCAGGCG 1440
AGCTTGGGGC CGAGTGAGCA TCCTGGCGCC TGGGAACCAT 1480
AGTGGCAGCA TGCTGGAACA GGAATCCACA GACCTGACAC 1520
AGGTGAGCGA GGCAGCCGGG CCCCTTGTCC CACCACCCGC 1560
ACTTCCCAAC TGCACAGCCT CGAGTAGAAC CCTGGATCCG 1600

1610 1620 1630 1640
GCTTGTTAAA GGACCAAAGG GCATCTAACA GCTTCTAGAC 1640
AGTGTGGCCC GAGGATCCCT GGGGTTATG CTTGAACGTT 1680
ACAGGGTTGA GGCTAAAGAC TGAGATTGAT TGTAGGGAAC 1720
CTCCAGTTAT TAAACGGTGC GGATTGCTAG AGGGTGGCAT 1760
AGTCCTTCAA TCCTGGCACC CGAAAAGCAG ATGCAGGAGC 1800

1810 1820 1830 1840
AGGAGCAGGA GCAAAGCCAG CCATGGAGTT TGAGGCCTGC 1840
TTGAACTACC TGAGATCCAA TAATAAACA TTTCATATGC 1880
TGTGAAAAA AAAAAAAAAA AAAA 1904

FIG. 2B

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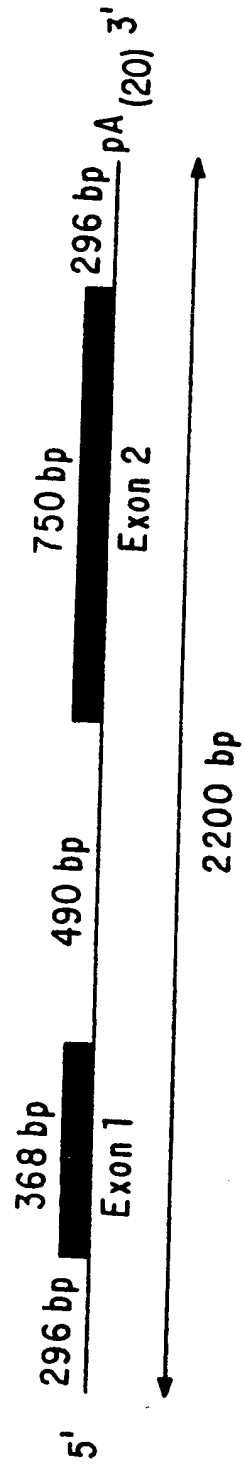


FIG. 3

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1 ATG AAT GGC TCC GGC AGC CAG GGC GCG GAG AAC ACG AGC CAG GAA GGC GGT AGC GGC GGC TGG CAG CCT GAG GCG 75
 1 M N G S G S Q Q A E N T S Q E G S G G W Q P E A 25
 76 GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC GTG GGC AAC GCG CTG GTG CTG GCG GTG CTG 150
 26 V L V P L F A L I F L V G T V G N A L V L A V L 50
 151 CTG CGC GGC GGC CAG GCG GTC AGC ACC AAC CTG TTC ATC CTC AAC CTG GGC GTG GGC GAC CTG TGT TTC ATC 225
 51 L R G Q A V S T T N L F I L N L G V A D L C F I 75
 226 TGC TGC TGC CCT TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TGC CTG CTC TGC AAG GCT 300
 76 L C C V P F Q A T I Y T L D W V F G S L L C K A 100
 301 GTT CAT TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC ACG CTG GCC GGC GTC TCC CTG GAC AG 368
 101 V H F L I F L T M H A S S F T L A A V S L D R 123
 369 gtaaggaccagaaacatccagatgccccggaggatcttgactggaaagacigaaatcctggtcgtgacatt
 449 agttccctgccccttcacatcacttgacattccacagaagcggtaagagcggtggtccttattctcctctggtt
 529 tccactgagtgcaacatgctgctgagtagcgtgaggactcacaatttcagcttctttaggagttccttgc
 609 gtagttgaccacagtccttccaggttctgtcagaccctcagcagggatcgcctccctggtgtcaccagag
 689 gataacaatcacgccccagaaatccagacagattctacaacttttagcttcggtgttgggggtgcccttcacgt
 769 ggagtaggtcggtggccacattccagaggtagacatagcctagcagtgatcctcgtctagctgagtcgccccact
 849 gtccccacag
 859 G TAT CTG GCC ATC CGC TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC ATC GCG 931
 124 Y L A I R Y P L H S R E L R T P R N A L A A I G 147
 932 CTC ATC TGG GGC CTA GCA CTG CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG 1006
 148 L I W G L A L L F S G P Y L S Y Y R Q S Q L A N L 50
 1007 ACA GTA TGC CAC CCA GCA TGG AGC GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC GTC TTT AGC TAC CTG 1081
 151 T V C H P A W S A P R R A M D L C T F V F S Y L 172
 1082 CTG CCA GTG CTA GTC CTC AGT CTG ACC TAT GCG CGT ACC CTG CGC TAC CTC TGG CGC ACA GTC GAC CCG GTG ACT 1156
 173 L P V L V L S L L I Y A R T L L R Y L W R T V D P V T 222

FIG.3A

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1157	GCA GGC TCA GGT TCC CAG CGC GCC AAA CGC AAG GTG ACA CGG ATG ATC ATC ATC GTG GCG GTG CTT TTC TGC CTC	1231
223	A G S G S Q R A K R K V T R M I I I I I V A V L F C L	247
1232	TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC GTG TGG TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG	1306
248	C W M P H H A L L I L L C V W F G R F P L T R A T Y A	272
1307	TTG CGC ATC CTT TCA CAC CTA GTT TCC TAT GCC AAC TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG	1381
273	L R I L S H L V S Y A N S C V N P I V Y A L V S K	297
1382	CAT TTC CGT AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG CTG CGC CCT GCC CCG AGG CGA GCT TCG GGC CGA GTG	1456
298	H F R K G F R K I C A G L L R P A P R A S G R V	322
1457	AGC ATC CTG GCG CCT GGG AAC CAT AGT GGC AGC ATG CTG GAA CAG GAA TCC ACA GAC CTG ACA CAG GTG AGC GAG	1531
323	S I L A P G N H S G S M L E Q E S T D L T Q V S E	347
1532	GCA GCC GGG CCC CTT GTC CCA CCA CCC GCA CTT CCC AAC TGC ACA GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT	1606
348	A A G P L V P P P A L P N C T A S S R T L D P A C	372
1607	TAA	1609
	*	

FIG.3B

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10 20 30 40
MNGSGSQGAE NTSQEGGSGG WQPEAVLVPL FFALIFLVGT 40
VGNALVLAVL LRGQAVSTT NLFTILNLGVA DLCFILCCVP 80
FQATTYTLDD WVFGSLCKA VHFLIFLTMH ASSFTLAAVS 120
LDRYLAI RYP LHSRELRTPR NALAAIGLIW GLALLFSGPY 160
LSYYRQSQLA NLTVCHPAWS APRRRAMDLC TFFVFSYLLPV 200
210 220 230 240
LVLSLT YART LRYLWRTVDP VTAGSGSQRA KRKVTRMIII 240
VAVLFCLCWM PHHALILCW FGRFPLTRAT YALRILSHLV 280
SYANSCVNPI VYALVSKHFR KGFRKICAGL LRPAPRRASG 320
RVSILAPGNH SGSMLEQEST DLTQVSEAAG PLVPPPALPN 360
CTASSRTLDP AC 372

FIG. 4

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ratgal1p	1	MELAPVNLSEGN	SDPP	EPPA	EP	RR	PL	FG	I	G	V	EN	F	33																					
ratgal2p	1	- - - - -	MNGSGSQGA	ENT	SQ	EG	SG	GW	Q	P	E	AV	26																						
ratgal1p	34	ITLVV	FG	L	I	F	A	M	G	V	L	G	N	S	L	V	I	T	V	L	A	R	S	K	P	G	K	P	R	66					
ratgal2p	27	LVPLF	F	E	A	L	I	F	L	V	G	T	V	G	N	A	L	V	L	A	V	L	R	G	-	-	G	Q	A	V	57				
ratgal1p	67	STTNLF	I	L	N	L	S	I	A	D	L	A	Y	L	L	F	C	I	P	F	Q	A	T	V	Y	A	L	P	T	99					
ratgal2p	58	STTNLF	I	L	N	L	G	V	A	D	L	C	F	I	L	C	C	V	P	F	Q	A	T	I	Y	T	L	D	D	90					
ratgal1p	100	WV	L	G	A	F	I	C	K	F	I	H	Y	F	F	T	V	S	M	L	V	S	I	F	T	L	A	A	M	S	V	D	R	132	
ratgal2p	91	WV	F	G	S	L	L	C	K	A	V	H	F	L	I	F	L	T	M	H	A	S	S	F	T	L	A	A	V	S	L	D	R	123	
ratgal1p	133	Y	V	A	I	V	H	S	R	R	S	S	S	L	R	V	S	R	N	A	L	L	G	V	G	F	I	W	A	L	S	I	A	M	165
ratgal2p	124	Y	L	A	I	R	Y	P	L	H	S	R	E	L	R	T	P	R	N	A	L	A	A	I	G	L	I	W	G	L	A	L	F	156	
ratgal1p	166	A	S	P	V	A	Y	Q	R	L	F	H	R	D	S	N	Q	T	F	C	W	E	H	W	P	N	Q	L	H	K	K	A	Y	198	
ratgal2p	157	S	G	P	Y	L	S	Y	R	Q	S	Q	L	-	A	N	L	T	V	C	H	P	A	W	S	A	P	-	R	R	R	A	M	187	
ratgal1p	199	V	V	C	T	F	V	F	G	Y	L	L	P	L	L	I	C	F	C	Y	A	K	V	L	N	H	L	H	K	K	L	K	N	231	
ratgal2p	188	D	L	C	T	F	V	F	S	Y	L	L	P	V	L	V	L	S	L	T	Y	A	R	T	L	R	Y	L	W	R	T	V	D	P	220

FIG. 5A

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ratgal1p	232	M	-	-	S	K	S	E	A	S	K	K	T	A	Q	T	V	L	V	V	V	F	G	I	S	W	L	P	H	H	262				
ratgal2p	221	V	T	A	G	S	G	S	Q	R	A	K	R	K	V	T	R	M	I	I	V	A	V	L	F	C	L	C	W	M	P	H	H	253	
ratgal1p	263	V	I	H	L	W	A	E	F	G	A	F	P	L	T	P	A	S	F	F	F	R	I	T	A	H	C	L	A	Y	S	N	S	S	295
ratgal2p	254	A	L	I	L	C	V	W	F	G	R	F	P	L	T	R	A	T	Y	A	L	R	I	L	S	H	L	V	S	Y	A	N	S	C	286
ratgal1p	296	V	N	P	I	I	Y	A	F	L	S	E	N	F	R	K	A	Y	K	Q	V	F	K	C	R	V	C	N	E	S	P	H	G	D	328
ratgal2p	287	V	N	P	I	V	Y	A	L	V	S	K	H	F	R	K	G	F	R	K	I	C	A	G	L	L	R	P	A	P	R	R	A	S	319
ratgal1p	329	A	K	-	-	-	-	-	-	-	-	E	K	N	R	I	D	T	P	P	S	T	N	C	T	H	V	-	-	-	-	-	-	346	
ratgal2p	320	G	R	V	S	I	L	A	P	G	N	H	S	G	S	M	L	E	Q	E	S	T	D	L	T	Q	V	S	E	A	A	G	P	L	352
ratgal2p	353	V	P	P	P	A	L	P	N	C	T	A	S	S	R	T	L	D	P	A	C													373	

FIG. 5B

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1	30
TGCGGACCACCACCAACTTGTACCTGGGCA	
	60
GCATGGCCGTGTCCGACCTACTCATCCTGC	
	90
TCGGGCTGCCGTTGACCTGTACCGCCTCT	
	120
GGCGCTCGCGGCCCTGGGTGTTGGGGCCGC	
	150
TGCTCTGCCGCCTGTCCCTCTACGTGGGCG	
	180
AGGGCTGCACCTACGCCACGCTGCTGCACA	
	210
TGACCGCGCTCAGCGTCGAGCGCTACCTGG	
	240
CCATCTGCCGCCCCGCTCCGCGCCCGCGTCT	
	270
TGGTCACCCGGCGCCGCGTCCGCGCGCTCA	
	283
TCGCTGTGCTCTG	

FIG.6

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gagctcgggaagcaggtacaagcgccactctccgcctgcgccgtggaatgcgcgccgggacc
antccgcagcccttccccagcgccgcccggccgctgctggggacaacctcgccctcctgtn
tcttgctcctcctcctgaccccagcgacccccatccccgccccagatgaggcaaggtcc
ctccgccttcagcccggcagagtgcactaggagttgcagcgccgcagccccgggagctt
cccgtcgcggagaccagacggctgcaggagcccgggcagcctcggggtcagcggcaccA
TGAACGTCTCGGGCTGCCAGGGGCCGGGAACGCGAGCCAGGCGGGCGGCGGGGAGGCTG
GCACCCCGAGGCGGTACGTGCCCCGTCTTCGCGCTCATCTTCCTCGTGGGCACCGTG
GGCAACACGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAGGCGGTACAGACTACCAACC
TGTTTCATCCTTAACCTGGGCGTGGCCGACCTGTGTTTCATCCTGTGCTGCGTGCCCTTCCA
GGCCACCATCTACACCTGGACGGCTGGGTGTTGCGCTCGCTGCTGTGCAAGGCGGTGCAC
TTCCTCATCTTCCTACCATGCACGCCAGCAGCTTCACGCTGGCCGCCGTCTCCCTGGACA
Ggtgagccagcgcttggcctccctgggagatgggcatccacgcgggggatggagcgggag
gcgggactggggaccaagaaggagcgcgagagtgggacaggacactaagaaggcagtgga
agacaagcgggcgcggaggaggaaaaagaggaataagaatgggggaccgtggtgtccctcg
gttagatgcgtcctggggcctggaagcctggagaatgtggctctccagcgccgccgtgcc
tgacaacgcgcagcgttcccagtagcgcgcttgtgcgcgttcattctcgcttgagctta
atgccctccgtgagggtaggataggacaaagtgcccaatatacagaagagttgagttccta
agtaactcgctcagagtcgccagccaagggatcgggtgcgttgaaagtaccgtctgtctcc
tgcagccaacttcaggcgcctccactgcgctcgctccaagccacgggttggttggttggt
gcagctggctcaggtccaggctgtggatccttgggtccttgaaggatccactccggagtc
ccagcgagcgtgcctaaaggtccctagctcagtcgccagccactctgcctctcgctccaa
acaaaaacaaaaacaaaaataaaatccaaaacaagtggggcgggagaggaagcgttgccctgg
ggttcttcccagccagaggagagcgaagagacgcacattcgggagagccgccgggact
caggtggagcttgaaaggacactgggatgggttccctggggaggaaatccgggtatttccc
ctctccatcctctggaaaaacagagaggcgaggccagactgccccacacctcctgtagcc
actgagcgcgaaagtgcgttggttccgagcgcgctgggtgggatccacaaagctcgcatctc
tcaggaatcccctgagaaattaactgtcccttgcccaacatgtcttctccaggctgtctgc
tagagcctcaggcgcctccgcccctccctcccgcgccaccgtcaccagtgggtagtcacagc
ctcccggagcccatagcgggttctccaacctttagcttctcagtggttgggggtgcctct
cagtgaggagactgtggttgagtcctccgggggcagcgggagaatggcttgaggcacacctt
tcttgctgccggccccgccccatttccagcgtccgctgagtgctgtgggacacgctgggaggc
ccccacctccgcccacgcccagcctcaccaccctcctctgtgtgcggtgtaaccatg
cgtaaggaccttccctgagagcagccttgggaccgaggtgcaggggtcgcggccctccag
catgaatgtgcccgtcagccgacgtctcccttcccgggtctgaccgcagGTATCTGGCCAT
CCGCTACCCGCTGCACTCCCGGAGCTGCGCACGCCTCGAAACGCGTGGCAGCCATCGGG
CTCATCTGGGGGCTGTCGCTGCTCTTCTCCGGGCCCTACCTGAGCTACTACCGCCAGTCGC

FIG. 7A

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AGCTGGCCAACTGACCGTGTGCCATCCCGCGTGGAGCGCCCTCGCCGCCGCGCCATGGA
CATCTGCACCTTCGTCTTCAGCTACCTGCTTCCTGTGCTGGTTCTCGGCCTGACCTACGCG
CGCACCTTGCGCTACCTCTGGCGCGCGTTCGACCCGGTGGCCGCGGGCTCGGGTGGCCGGC
GCGCCAAGCGCAAGGTGACACGCATGATCCTCATCGTGGCCGCGCTCTTCTGCCTCTGCTG
GATGCCCCACCACGCGCTCATCCTCTGCGTGTGGTTGGCCAGTTCCTGCTCACGCGCGCC
ACTTATGCGCTTCGCATCCTCTCGCACCTGGTCTCCTACGCCAACTCCTGCGTCAACCCCA
TCGTTTACGCGCTGGTCTCCAAGCACTTCGCAAAGGCTTCGCGACGATCTGCGCGGGCCT
GCTGGGCCGTGCCCCAGGCCGAGCCTCGGGCCGTGTGTGCGCTGCCGCGCGGGGCACCCAC
AGTGGCAGCGTGTGGAGCGCGAGTCCAGCGACCTGTTGCACATGAGCGAGGCGGCGGGGG
CCCTTCGTCCCTGCCCCGGCGCTTCCAGCCATGCATCCTCGAGCCCTGTCTGGCCCGTC
CTGGCAGGGCCCCAAAGGCAGGCGACAGCATCCTGACGGTTGATGTGGCTGAaagcactta
gcgggcgcgctgggatgtcacagagttggagtcattgttggggaccgtggggagagcttt
gcctgttaataaaaacgcacaaaccatttcacacacagtgacagcgctgtttcgcgtttctc
attgtcttgagattctgggaggaagcctctggggcttcacagaggggctccctaggggtaa
gtgcaggaccctttgcagagctaccaggaaagagggtgatcacacctcaggcagccgggt
tacaatccgcataaaaaatctgagtcctggggagcggtgcgacagaggcaggcagattgtttaa
ggcgttcgataaagtcggttgatgacagacacagatgtgtgttcccagccgcatttgtgct
ctgggtgtgtgacaggctctgtccttgctgtttcagctcccagggccccctttgagtcctggg
cagcccagtcagtcctccgtccatttttggccttagcttttccttccttggtacatctggg
ccaggatcaagtctccagcagctgtttcactcccc

FIG. 7B

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ATGAACGTCTCGGGCTGCCAGGGGCGGGGAACGCGAGCCAGGCGGGCGGCGGGGGAGGCT
GGCACCCCGAGGCGGTATCGTGCCCTGCTCTTCGCGCTCATCTTCCTCGTGGGCACCGT
GGGCAACACGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAGGCGGTGAGCACTACCAAC
CTGTTTCATCCTTAACCTGGGCGTGGCCGACCTGTGTTTCATCCTGTGCTGCGTGCCCTTCC
AGGCCACCATCTACACCCTGGACGGCTGGGTGTTGCGCTCGCTGCTGTGCAAGGCGGTGCA
CTTCCTCATCTTCCTCACCATGCACGCCAGCAGCTTCACGCTGGCCGCGGTCTCCCTGGAC
AGGTATCTGGCCATCCGCTACCCGCTGCACTCCCGCGAGCTGCGCACGCCTCGAAACGCGC
TGGCAGCCATCGGGCTCATCTGGGGGCTGTGCTGCTCTTCTCCGGGCCCTACCTGAGCTA
CTACCGCCAGTCGCAGCTGGCCAACCTGACCGTGTGCCATCCCGCGTGGAGCGCCCTCGC
CGCCGCGCCATGGACATCTGCACCTTCGTCTTCAGCTACCTGCTTCCTGTGCTGGTTCTCG
GCCTGACCTACGCGCGCACCTTGCGCTACCTCTGGCGCGCCGTGACCCGGTGGCCGCGGG
CTCGGGTGCCCGGCGCGCAAGCGCAAGGTGACACGCATGATCCTCATCGTGGCCGCGCTC
TTC.TGCCTCTGCTGGATGCCCCACCACGCGCTCATCCTCTGCGTGTGGTTCGGCCAGTTCC
CGCTCACGCGCGCCACTTATGCGCTTCGCATCCTCTCGCACCTGGTCTCCTACGCCAACTC
CTGCGTCAACCCCATCGTTTACGCGCTGGTCTCCAAGCACTTCGCAAAGGCTTCGCGACG
ATCTGCGCGGGCCTGCTGGGCGGTGCCCCAGGCCGAGCCTCGGGCCGTGTGTGCGCTGCCG
CGCGGGGCACCCACAGTGGCAGCGTGTGGAGCGCGAGTCCAGCGACCTGTTGCACATGAG
CGAGGCGCGGGGGCCCTTCGTCCCTGCCCCGGCGCTTCCAGCCATGCATCCTCGAGCCC
TGTCTTGGCCCGTCTTGGCAGGGCCCAAAGGCAGGCGACAGCATCCTGACGGTTGATGTGG
CCTGA

FIG.8

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gca	Ala(A)	2	#	cag	Gln(Q)	8	#	uug	Leu(L)	3	#	uaa	Ter(.)	0
gcc	Ala(A)	23	#	---	Gln(Q)	8	#	---	Leu(L)	56	#	uag	Ter(.)	0
gcg	Ala(A)	19	#	gaa	Glu(E)	0	#	aaa	Lys(K)	1	#	uga	Ter(.)	1
gcu	Ala(A)	2	#	gag	Glu(E)	6	#	aag	Lys(K)	5	#	---	Ter(.)	1
---	Ala(A)	46	#	---	Glu(E)	6	#	---	Lys(K)	6	#	aca	Thr(T)	1
aga	Arg(R)	0	#	gga	Gly(G)	1	#	aug	Met(M)	6	#	acc	Thr(T)	10
agg	Arg(R)	1	#	ggc	Gly(G)	25	#	---	Met(M)	6	#	acg	Thr(T)	6
cga	Arg(R)	2	#	ggg	Gly(G)	7	#	uuc	Phe(F)	17	#	acu	Thr(T)	2
cga	Arg(R)	19	#	ggu	Gly(G)	1	#	uuu	Phe(F)	0	#	---	Thr(T)	19
cgg	Arg(R)	2	#	---	Gly(G)	34	#	---	Phe(F)	17	#	ugg	Trp(W)	8
cgu	Arg(R)	3	#	cac	His(H)	10	#	cca	Pro(P)	4	#	---	Trp(W)	8
---	Arg(R)	27	#	cau	His(H)	1	#	ccc	Pro(P)	10	#	uac	Tyr(Y)	10
aac	Asn(N)	9	#	---	His(H)	11	#	ccg	Pro(P)	4	#	uau	Tyr(Y)	2
aau	Asn(N)	0	#	aua	Ile(I)	0	#	ccu	Pro(P)	4	#	---	Tyr(Y)	12
---	Asn(N)	9	#	auc	Ile(I)	18	#	---	Pro(P)	22	#	gau	Val(V)	0
gac	Asp(D)	7	#	auu	Ile(I)	0	#	agc	Ser(S)	11	#	guc	Val(V)	9
gau	Asp(D)	1	#	---	Ile(I)	18	#	agu	Ser(S)	1	#	gug	Val(V)	18
---	Asp(D)	8	#	cua	Leu(L)	0	#	uca	Ser(S)	0	#	guu	Val(V)	3
ugc	Cys(C)	14	#	cuc	Leu(L)	17	#	ucc	Ser(S)	9	#	---	Val(V)	30
ugu	Cys(C)	2	#	cug	Leu(L)	32	#	ucg	Ser(S)	7	#	nnn	???(X)	0
---	Cys(C)	16	#	cuu	Leu(L)	4	#	ucu	Ser(S)	0	#	TOTAL		388

FIG.9A

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MNVSGCPGAGNASQAGGGGWHPEAVIVPLL FALIFLVGTVGNTL
VLAVLLRGGQAVSTTNLFILNLGVADLCFILCCVPFQATIYTLDGWV
FGSLLCKAVHFLIFLTMHASSFTLAAVSLDRYLAI RYPLHSREL RTPR
NALAAIGLIWGLSLLFSGPYLSYYRQSQLANLTVCHPAWSAPRRRA
MDICTFVFSYLLPVLVLGLTYARTLRYLWRAVDPVAAGSGARRAK
RKVTRMILIVAALFCLCWMPHHALILCVWFGQFPLTRATYALRILS
HLVSYANSCVNP I VYALVSKHFRKGFRTICAGLLGRAPGRASGRVC
AAARGTHSGSVLERESSDLLHMSEAAGALRPCPGASQPCILEPCPGP
SWQGPKAGDSILTVDVA

FIG.9B

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Pharmacology of Human and Rat GALR2 IC ₅₀ (nM)			
PEPTIDE	hGALR2	rat GALR2	hGALR1*
human galanin	3.8 ± 0.28	1.5 ± 0.45	0.13 ± 0.04
porcine galanin	1.5 ± 0.03	0.83 ± 0.5	0.14 ± 0.04
rat galanin	1.6 ± 0.42	0.9	0.1
rat Gal (2-29)	15.4 ± 7.9	2.9 ± 0.9	17 ± 7.5
rat Gal (3-29)	>1000	>1000	>1000
M40	9.5 ± 0.7	1.8 ± 1.8	0.48 ± 0.2
M35	5.6 ± 0.2	0.43 ± 0.18	0.04 ± 0.02
C7	40.5 ± 19	13.5 ± 0.7	6.3 ± 6.7
Kd	5 nM	0.19 nM	0.07 nM

FIG.10

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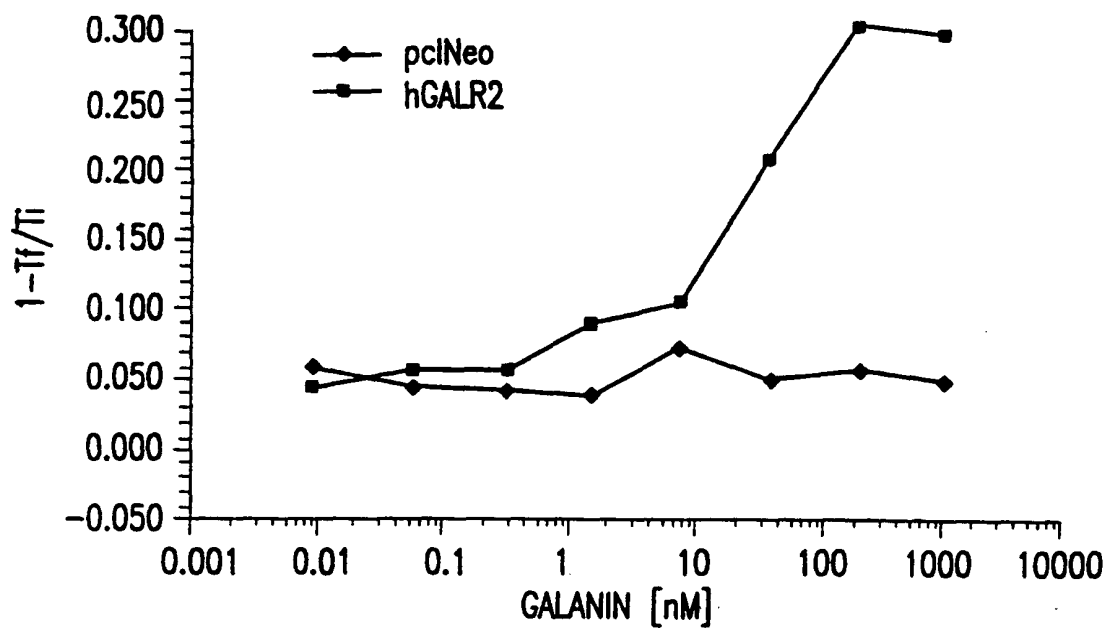


FIG.11A

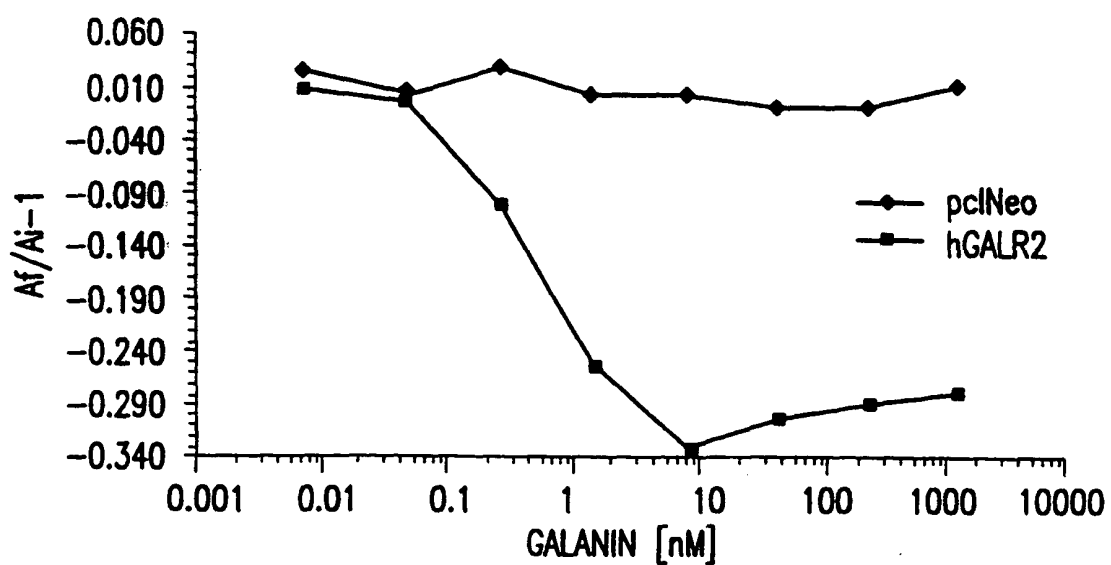


FIG.11B

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gccctttccactttggtgataccATGAATGGCTCGGACAGCCAGGGGGCGGA
GGACTCGAGCCAGGAAGGTGGCGGCGGCTGGCAGCCCGAGGCG
GTCCTCGTACCCCTATTTTTTCGCGCTCATCTTCCTCGTGGGCGCTG
TGGGCAACGCGCTGGTGCTGGCGGTGCTGCTGCGCGGCGGCCAG
GCGGTCAGCACCAAGAACCTATTCATCCTCAACCTGGGTGTGGC
CGACCTGTGTTTCATCCTGTGCTGCGTGCCTTTCCAGGCCACCATC
TATACCCTGGACGATTGGGTGTTTGGCTCACTGCTCTGCAAGGCC
GTTCAATTCCTCATCTTCCTCACTATGCACGCCAGCAGCTTCACGC
TGGCCGCTGTCTCGCTGGACAGgtgagtgaacattctgtggtgtctgagaactgggt
accaggttaggagcttgactggagtcgccacgcaaggatccagaagggatgcgtagtcggggag
aacactaaaattacaaagtggcccgaggccgtgaaacgcaaggggaaaggggactaagactccg
tgactaagagtgctcccttgattaagtcggtcctcagacctcgaaggctggagaaatcggtattctgggg
tctttacgttattgttgcttgagctaaaagtctctcagaaacattgcagtactcagaccagagttggcttg
caaagtaacttgccagatttcaaagtctaattgagagctgcagagaggcatttgcttcttgccccaag
ctcagcacctggagcgttgctccggctttaggcttaggactgagctgtactttggatagaccatgtctga
agtccaaggcagcgggagtgagggctcctagcggacgtctaagccttcaggccaaggctccccg
cccgagacgcctgcggttgatgttccttcctagctaaaggaccagaaagagaaaacttcagaat
gctctgaaggactcgtgactggaaaagacactagaaacaggctcctgggaaggatgtcattagttccc
tgcccttcgcatcacttgcccttcccacagtagagcgtgaagagaggcggagatcctcattctctg
ctttccactgagtgcaacatgtgggttctgagtcgctggtgggacgcacaaaacttcagctttcttcag
ggatttccttgctctacccaagtcttctccgggttgctgtcagagagcctcaggcattagagatttgctc
cctcggttgctcacaagaggataataatcactgccccagaagtcttgcatattctacaacttttagtttt
cggtgggttggggatgcccttctcgctggtaggtcagtgccacattctcagggttggtaatggtctagc
agtgaattagtgaaatcctttcgcttacctgtcgtcgtcgtccccccgccccactgtccactcagGTAT
CTGGCCATCCGCTACCCGATGCACTCCCGAGAGTTGCGCACACCT
CGAAACGCGCTGGCGGCCATCGGGCTCATCTGGGGGCTAGCACT
GCTCTTCTCCGGGGCCCTACCTGAGCTACTACAGTCAGTCGCAGCT
GGCCAATCTGACGGTGTGCCACCCAGCGTGGAGCGCACCCAGAC
GTCGCGCCATGGACCTCTGCACTTTTGTCTTTAGCTACCTGTTGCC
AGTGCTGGTGCTCAGCCTGACCTATGCGCGCACCCCTGCACTACCT
CTGGCGCACAGTTGACCCAGTAGCTGCAGGCTCAGGTTCCCAGC
GCGCCAAGCGCAAGGTGACACGGATGATCGTCATCGTGGCGGTA
CTCTTCTGCCTCTGTTGGATGCCCCACCAAGCGCTTATCCTCTGCG
TGTGGTTTGGTCGCTTTCCGCTCACGCGTGCCACTTACGCCCTGC
GCATCCTTTACATCTAGTATCTTATGCCAACTCGTGTGTCAACCC
CATCGTTTATGCTCTGGTCTCCAAGCATTTCGCAAAGGTTTCCG
CAAAATCTGCGCGGGCCTGCTACGCCGTGCCCCGAGGAGAGCTT
CAGGCCGAGTGTGCATCCTGGCGCCTGGAACCATAGTGGTGGC
ATGCTGGAACCTGAGTCCACAGACCTGACACAGGTGAGCGAGG
CAGCCGGGCCCCCTCGTCCCCGCACCCGCACTTCCCAACTGCACA
ACCTTGAGTAGAACCTCGATCCAGCCTGTTAAaggaccaaagggcatct
aacagcttctaaggcgga

FIG. 12

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MNGSDSQGAEDSSQEGGGGWQPEAVLVPLFFALIFLVGAVGNALVL
AVLLRGGQAVSTTNLFILNLGVADLCFILCCVPFOATIIYTLDDWVFG
SLLCKAVHFLIFLTMHASSFTLAAVSLDRYLAI RYPMHSRELRTPRN
ALAAIGLIWGLALLFSGPYLSYYSQSQLANLTVCHPAWSAPRRRAM
DLCTFVFSYLLP VLVLSLTYARTLHYLWRTVDPVAAGSGSQRAK RK
VTRMIVIVAVLF CLCWMPHHALILCVWFGRFPLTRATYALRILSHL
VSYANSCVNP I VYALVSKHFRKGFRKICAGLLRRAPRRASGRVCIL
APGNHSGGMLEPESTDLTQVSEAAGPLVPAPALPNCTTLSRTLDPAC

FIG.13